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L5: Entry 9 of 18 File: USPT Aug 29, 1995

DOCUMENT-IDENTIFIER: US 5445958 A
TITLE: Process for purifying blood clotting factors

BSPR:
Further significant plasma proteins, which may be considered to fall within the general category of blood clotting factors, include Protein C and Protein S. Protein C is an inactive zymogen of the proteolytic enzyme Activated Protein C (APC,PC.sub.a), Unlike the above-mentioned clotting factors, APC has an anticoagulant effect which functions through the proteolysis of Factor V and Factor VIII which are thus inactivated. Activation of Protein C appears to be by a feedback process involving thrombin and an endothelial-bound protein, thrombomodulin, which both function during the coagulation process. The activity of APC is then enhanced by the presence of Protein S, which seems to act as a cofactor.

BSPR:
It has been found that at relatively high ionic strengths, the binding to the chelate of prothrombin (Factor II) and thrombin is reduced, while that of Factors IX and X and Protein C is increased. Since, in general, it is desirable to reduce the prothrombin level in concentrates of the other blood clotting factors, in order to avoid the possibility of thrombin formation, this finding is particularly useful. It is thus preferred to load the metal chelate in an aqueous solution of relatively high ionic strength, for example containing 0.4 to 1.0M, preferably 0.4 to 0.6M , most preferably about 0.5M, sodium chloride and/or one or more other electrolytes providing a solution of equivalent ionic strength.

BSPR:
After elution, the solution may be freeze dried. For inactivation of virus infections, the freeze dried concentrate may be heated at, for example, 80.degree. C. We have found that the purified concentrates according to the invention substantially survive such heating, avoiding significant activation of the respective factors to a greater extent than previous concentrates.

BSPV:
TGt 50: Measures the time taken to generate a known amount of thrombin. It is a measure of the activation of clotting factors which operate earlier in the process.

DEPR:
Purification of Protein C is complicated by co-elution of prothrombin and Factor X in conventional chromatographic systems

prothrombin and Factor X in conventional chromatographic systems such as heparin-Sepharose or Dextran-Sulphate-Sepharose. However, in these systems, Factor IX contamination is minimal due to tighter binding of that protein. The metal chelate system described here can therefore be used as a step in the purification of Protein C.

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USPT,JPAB,EPAB,DWPI	l7 and (DEAE or Sephadex)	3	L8
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USPT,JPAB,EPAB,DWPI	prothrombin and readsorbtion	0	L6
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